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Making New Connections: Role of ERK/MAP Kinase Signaling in Neuronal Plasticity

Minireview

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One of the most intriguing questions in neurobiology is how neurons and synapses encode the long-term changes in synaptic efficacy that underlie memory consolidation. A recent set of studies suggests that the ERK/mitogen-activated protein kinase (MAPK) plays a fundamental role in vertebrate and invertebrate memory consolidation. These findings suggest that the ERK/MAPK cascade should join the cAMP response element binding protein/cAMP response element (CREB/CRE) and cAMP/protein kinase A (PKA) pathways as evolutionarily conserved regulators of memory formation. Because the MAPK cascade was previously identified as a critical regulator of cell growth, its regulatory role in synaptic function in postmitotic neurons was unexpected. Nevertheless, a wide body of work now shows that the MAPK cascade is potently activated by synaptic activity and by increases in intracellular Ca^{2+} and cAMP. Here, we focus on recent exciting studies that indicate that the MAPK signaling cascade is critical for both memory consolidation and long-term neuronal plasticity. We also address the mechanisms for activity- and plasticity-mediated activation of MAPK in central nervous system (CNS) neurons and discuss evidence that activation of CREB by the ERK/MAPK cascade plays a pivotal role in some forms of neuronal plasticity.

Localization and Regulation of MAPK Suggest a Role in Neuronal Plasticity

The MAPK cascade is an evolutionarily conserved signaling cassette that plays a critical role in cell growth and survival in yeast, plants, and vertebrates. Interestingly, in vertebrates at least seven MAPK cascades have been identified. In some cases, specialized physiological roles have been identified for these regulatory cascades. For example, p38 and jun kinase (JNK) are stress-activated protein kinases that play a role in the inflammatory response and regulate neuronal survival (Xia et al., 1995). On the other hand, the ERK/MAPK cascade plays a central role in regulating proliferation and differentiation. For example, the ability of growth factors to promote cell growth typically depends on the activation of receptor tyrosine kinases, which recruit Ras family small G proteins and lead to the sequential activation of Raf (MAPK kinase kinase), MEK (MAPK kinase), and ERK/MAPK (reviewed by Seger and Krebs, 1995; see also Figure 1).

The sequential nature of the ERK/MAPK cascade provides multiple points at which the response can be regulated by phosphorylation and dephosphorylation and allows for tremendous amplification of extracellular signals. Because the duration of MAPK activation can determine whether nuclear or cytosolic targets are activated and whether proliferation or differentiation occurs, this allows for a spatially and temporally graded response. The activation and nuclear translocation of MAPK plays an essential role in the inducible expression of many immediate-early and late-response genes.

Interestingly, Ras/MAPK signaling components are highly enriched in the adult CNS, and expression of many MAPK regulators (N-Shc, RasGRF, RasGRP, SynGAP, Ca^{2+}/DAG GTP exhange factors [GEFs], NF1, N-Ras, and B-Raf) is largely restricted to the CNS. An initial clue to the role of the ERK/MAPK cascade in the CNS came from the observation that expression of MAPK signaling components is especially high in associational areas implicated in learning and memory, such as the hippocampus, neocortex, and cerebellum. Because long-term neuronal plasticity and memory consolidation require de novo gene expression, the prominent role of MAPK in inducible gene expression supports the idea that MAPK may regulate synaptic plasticity and long-term memory (LTM) formation. The ability of the MAPK cascade to integrate coincident signals and to translate the magnitude of signaling into a temporally and spatially graded response further suggests a role in long-term adaptive plasticity in the CNS.

How Is MAPK Activated by Synaptic Activity?

Synaptic activity-induced increases in intracellular Ca^{2+} potently activate MAPK in CNS neurons. However, the mechanisms by which Ca^{2+} activates MAPK in CNS neurons are not well defined. In PC12 cells, activation of Ras and the tyrosine kinase Src is required for Ca^{2+} stimulation of MAPK (Rosen et al., 1994; Rusanescu et al., 1995). Consequently, several mechanisms have been proposed for the activation of Ras by Ca^{2+}, including Ca^{2+}/calmodulin (CaM) stimulation of the GEF RasGRF, CaM kinase II inhibition of the GTPase activating protein SynGAP (Chen et al., 1998), and activation of Pyk2 or Fak tyrosine kinases. Recently, several additional Ca^{2+}-regulated Ras GEFs (RasGRP and Ca^{2+}/DAG GEFs) have been characterized. Additional work is required to define which Ras/MAPK regulators actually mediate responsiveness to Ca^{2+} in CNS neurons.

Activity-generated Ca^{2+} influx in CNS neurons also markedly increases intracellular cAMP via activation of Ca^{2+}/CaM-sensitive adenylyl cyclases. The ability of the small G protein Rap1 to couple increases in intracellular cAMP to activation of MAPK suggests another potential mechanism for the activation of MAPK by Ca^{2+} (Vossler et al., 1997). The activation of Rap1 by cAMP may be mediated by a recently reported family of GEFs that directly bind cAMP (Kawasaki et al., 1998). Consistent with these studies, activators of adenylyl cyclase markedly increase MAPK activity in hippocampal neurons (Martin et al., 1997; Impey et al., 1998). Thus, in addition to being activated by Ca^{2+}, MAPK may also be a major cAMP-stimulated kinase in the CNS. These studies raise the possibility that some of the effects of cAMP, which have been attributed to PKA, may be the result of MAPK activation. For example, some of the inhibitors used to implicate PKA (e.g., cAMP analogs) may regulate MAPK.

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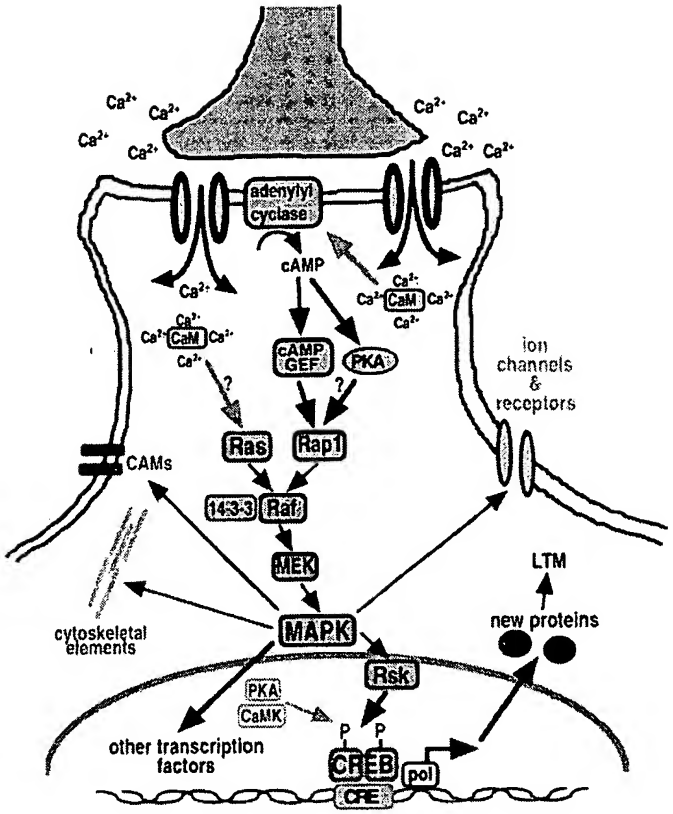

Figure 1. A Model for the Activation of ERK/MAPK by Synaptic Activity and Its Potential Regulatory Targets

Release of an excitatory neurotransmitter onto a bouton depolarizes a neuron resulting in the influx of Ca^{2+} through ionotropic receptors or voltage-gated Ca^{2+} channels. Ca^{2+} activates Ras family G proteins though an unknown mechanism, possibly involving Src family tyrosine kinases. The association of Ras and Raf with 14-3-3 proteins then induces the phosphorylation and activation of Raf. Alternatively, Ca^{2+}/CaM can stimulate adenylyl cyclases resulting in the accumulation of cAMP, which in turn activates Rap1 via cAMP GEFs or PKA. Rap1 may then activate Raf. The activation of Raf leads to the sequential phosphorylation and activation of MEK and MAPK. Activated MAPK likely has multiple targets, including CREB, which mediate its ability to induce long-term adaptive changes in neurons. The resulting synthesis of new proteins mediates the long-term remodeling of the synapse believed to underlie LTM and L-LTP. Other potential targets of MAPK are cell adhesion molecules (CAMs), cytoskeletal elements, and ion channels.

A Role for ERK/MAPK in Neuronal Plasticity

The first evidence for a role for the MAPK cascade in invertebrate neuronal plasticity came from studies of long-term facilitation (LTF) in *Aplysia*, a model for long-term sensitization of the gill withdrawal reflex. LTF is produced by exposing sensory–motor neuron synapses to multiple spaced pulses of serotonin, thus mimicking the release of serotonin by an interneuron in vivo. Kandel and coworkers have shown that the transcription factors ATF4 and C/EBP regulate LTF. Because ATF4 and C/EBP are thought to be phosphorylated by MAPK, the role of MAPK in LTF was examined (Martin et al., 1997). MAPK is activated and translocated to the nucleus following LTF of *Aplysia* sensory neurons (Martin et al., 1997). When MAPK is selectively inhibited in sensory neurons by injection of an inactivating antibody, LTF is attenuated. Since short-term facilitation is not affected, these data suggest that MAPK is required specifically for LTF. Similarly, pharmacological inhibition of MEK also prevents the acquisition of LTF but not short-term facilitation. Taken together, these results suggest that MAPK activation and nuclear translocation induce the transcription and expression of new proteins necessary for LTF.

Long-term potentiation (LTP) is an activity-dependent strengthening of synaptic efficacy that has been proposed to be a cellular model for vertebrate memory formation. LTP has a short-term form that depends on the activation of kinases and phosphatases (decremental LTP or E-LTP) and a long-term form that requires de novo mRNA transcription and protein synthesis (L-LTP). L-LTP shares several mechanistic characteristics with LTM, including a requirement for CREB-dependent gene expression, as well as Ca^{2+}- and cAMP-dependent signaling.

Recent research indicates that MAPK signaling plays an important role in the induction of LTP. For example, stimuli that induce LTP in area CA1 of the hippocampus also potently activate MAPK (English and Sweatt, 1996; Impey et al., 1998), and pharmacological inhibition of MEK, an upstream activator of MAPK, partially inhibits LTP formation in area CA1 of the hippocampus (English and Sweatt, 1997). In addition to regulating short-term synaptic strength, treatment with the MEK inhibitor PD98059 completely blocks gene expression–dependent L-LTP (Impey et al., 1998). Thus, MAPK signaling not only regulates short-term synaptic function but may also promote the transcription and translation of new proteins required for L-LTP.

Activation of MAPK Is an Essential Step for Memory Formation

The *Drosophila* olfactory learning mutant, *leonardo*, strongly suggests a role for MAPK in invertebrate memory formation (Skoulakis and Davis, 1996). *leonardo* encodes a 14-3-3 family protein that is highly expressed in the mushroom bodies, the locus of *Drosophila* associative olfactory learning. 14-3-3 proteins bind directly to Raf and are critical for the activation of Raf by Ras. Decreased levels of *leonardo* expression in *Drosophila* result in impaired Ras/MAPK signaling. Moreover, *leonardo* mutants show a marked deficit in both short-term and long-term associative olfactory memory formation.

Several transgenic mouse mutants have also implicated the MAPK pathway in vertebrate LTM formation. Mice deficient for RasGRF, an activator of Ras, show a marked deficiency in LTP in the basolateral amygdala (Brambilla et al., 1997), a structure believed to help encode certain forms of associative fear conditioning. Therefore, Brambilla et al. (1997) examined cued fear conditioning in RasGRF-deficient mice. In cued fear conditioning, mice learn to fear a normally innocuous auditory tone that has been paired with aversive foot shock during training. LTM for cued fear conditioning is markedly compromised in RasGRF mutant mice. Immediate learning and short-term memory (STM) in RasGRF-deficient mice are unchanged relative to wild-type mice. Although Ras can potentially couple to other kinase cascades, RasGRF is a potent activator of ERK/MAPK signaling. Thus, these data suggest a role for the Ras/MAPK cascade in LTM formation. Interestingly, cued fear conditioning induces associative LTP in the lateral amygdala in vivo and in vitro. It is tempting to hypothesize that a possible role of Ras/MAPK activation in fear conditioning is to integrate associative inputs from the thalamus as well as the auditory cortex and induce an LTP-like increase in synaptic efficacy.

A study by Silva and colleagues also implicates the Ras/MAPK cascade in vertebrate memory consolidation; heterozygous neurofibramatosis type 1 (NF1) mutant mice have partial deficits in hippocampus-dependent spatial memory (Silva et al., 1997). In contrast to RasGRF, NF1 is a neuronal Ras GTPase-activating protein and functions as an inhibitor of Ras. Thus, this work implies that increased Ras activity perturbs memory formation. This study suggests that a dynamic balance of Ras activation is essential for memory formation in mice. Interestingly, these findings are reminiscent of earlier work in *Drosophila*, in which genetic mutations that increase cAMP levels (*dunce* phosphodiesterase mutant) or decrease cAMP levels (*rutabaga* adenylyl cyclase mutant) both disrupt learning and memory.

A caveat of gene disruption studies is that memory deficits can stem from developmental or compensatory changes in synaptic function or neuronal circuitry. Several recent studies avoid this problem by utilizing selective pharmacological inhibitors of MEK. The first study reports that a hippocampus-dependent learning paradigm, contextual fear conditioning, induces a marked activation of MAPK in the rat hippocampus (Atkins et al., 1998). Contextual fear conditioning is similar to the cued fear conditioning paradigm used by Brambilla et al. (1997) with the exception that the rats learn to associate an aversive foot shock with a novel "context" or spatial environment. Importantly, the increase in MAPK activity following contextual fear conditioning is not associated with the handling of the rats or the footshock. Thus, MAPK is specifically activated by the associative conditioning that induces memory consolidation rather than the aversive stimulus. Atkins et al. (1998) also examined whether MAPK activity is required for LTM for the contextual association. The systemic injection of SL327, at a dose that blocks conditioning-associated MAPK activation, attenuated contextual LTM formation. Because the effect of SL327 on STM was not assessed, it is not clear whether contextual LTM per se required MAPK signaling. Interestingly, SL327 also blocks hippocampal LTP. Taken in the context of work showing that MAPK activity is necessary for LTP, the study of Atkins et al. (1998) provides powerful evidence that MAPK signaling is an essential step in hippocampus-dependent LTM consolidation and supports the hypothesis that LTP and memory consolidation share similar mechanisms.

Atkins et al. (1998) also demonstrated that systemic injection of SL327 blocks cued fear conditioning in rats, suggesting that MAPK activity is required for amygdala-dependent memory formation. This observation supports the idea that the deficit in amygdala-dependent memory formation in RasGRF mutant mice is the result of impaired MAPK signaling. However, because of the systemic administration of SL327, Atkins et al. (1998) do not identify the anatomical region that requires MAPK activity for memory consolidation. It is conceivable that increases in MAPK activity in regions other than the hippocampus or amygdala contribute to memory formation.

A recent study using the Morris water maze spatial learning paradigm addressed the issue of anatomical specificity by infusing a MEK inhibitor selectively into the hippocampal formation (Blum et al., 1999). Training increased MAPK phosphorylation specifically in the dorsal hippocampus, an anatomical region required for Morris water maze spatial learning. Blum et al. (1999) also found that infusion of a MEK inhibitor reduced MAPK activity specifically in the dorsal hippocampus. Importantly, infusion of the MEK inhibitor attenuated the expression of LTM but was without effect on STM or on hippocampus-independent memory formation. Taken together, the work of Brambilla et al. (1997) and Blum et al. (1999) argues that MAPK signaling is a crucial regulator of LTM formation in vertebrates.

Additional evidence implicating the MAPK pathway in LTM formation comes from the study of conditioned taste aversion in rats (Berman et al., 1998). Conditioned taste aversion learning is produced by the pairing of a novel taste with a noxious stimulus. The injection of a different selective MEK inhibitor, PD98059, specifically into the "taste" or insular cortex impairs LTM for conditioned taste aversion, while PD98059 injection into an adjacent structure is without effect. However, interpretation of this data is complicated because MAPK is activated by exposure to a novel taste and therefore may play a more general role in differential taste perception. Nevertheless, the sensory representation of a novel taste is intact in the PD98059-treated rats because STM is not affected. The spatial as well as temporal specificity of MAPK inhibition demonstrated by Berman et al. (1998) argues that activation of MAPK signaling in the insular cortex contributes to LTM for taste aversion.

CREB Is a Major Target of MAPK Signaling

Although these recent genetic and behavioral studies implicate MAPK in LTM consolidation, the regulatory targets of MAPK during consolidation have not been defined. Each of the forms of neuronal plasticity and learning discussed above also require functional CREB. Thus, a likely candidate target of MAPK is CREB. Although CREB is not directly phosphorylated by MAPK, it is phosphorylated and transactivated by the MAPK-activated Rsk family of protein kinases (Xing et al., 1996; Impey et al., 1998). In particular, Rsk2 is required for Ca^{2+}-stimulated CREB phosphorylation in PC12 cells (Impey et al., 1998). However, multiple kinases including

Rsk1, Rsk2, Rsk3, and CaM kinase IV can promote activity-dependent CREB phosphorylation in hippocampal neurons (Impey et al., 1998). The identity of kinases that are required for activity-dependent CREB phosphorylation in vivo is still uncertain. Nevertheless, a number of recent studies indicate that CREB is a major target of MAPK during neuronal plasticity.

Gene expression–dependent L-LTP is attenuated in CREB mutant mice, and consequently L-LTP is thought to require CREB-dependent gene expression. Both L-LTP and L-LTP-associated CREB-dependent gene expression are attenuated by perfusion of hippocampal slices with the MEK inhibitor PD98059 (Impey et al., 1998). Because tetanus-induced CREB phosphorylation also requires MAPK signaling, a MAPK-activated CREB kinase, such as Rsk2, may be the indirect target of MEK inhibition. MAPK is also implicated in activity-dependent gene expression by a stimulus paradigm that elicits LTP in the striatum (Sgambato et al., 1998), where stimulation of corticostriatal afferents results in the activation of MAPK and phosphorylation of CREB. Both CREB phosphorylation and the induction of CREB-regulated genes (c-*fos*, *egr-1*, and MKP-1) are selectively blocked by infusion of PD98059 into the striatum.

A recent study also implicates the MAPK-activated CREB kinase, Rsk2, in cognitive dysfunction in humans. Inactivating Rsk2 mutations lead to Coffin-Lowry Syndrome, which is characterized by mental retardation and cranial dysmorphism. A defect in CREB phosphorylation is a potential cause of the cognitive impairment, because cells derived from patients with Coffin-Lowry Syndrome are deficient in growth factor–stimulated CREB phosphorylation (De Cesare et al., 1998).

Other Targets of MAPK during Memory Consolidation

The robust translocation of MAPK during synaptic plasticity (Martin et al., 1997; Impey et al., 1998; Sgambato et al., 1998) indicates that there are likely additional nuclear targets of MAPK signaling other than CREB. For example, several recent reports suggest that the transcription factor Elk1 is a major nuclear target of MAPK during synaptic plasticity and memory consolidation (Berman et al., 1998; Sgambato et al., 1998).

The prominent dendritic localization of activated MAPK following synaptic activity (Impey et al., 1998) suggests that it may also have important cytosolic targets. The best example of such a target is the *Aplysia* cell adhesion molecule ApCAM. MAPK activity is required for the downregulation and internalization of ApCAM, a key step in the induction of LTF. This is an important observation because the *Drosophila* (Fas II) and murine (NCAM) homologs of ApCAM have also been implicated in neuronal plasticity.

Collectively, these studies indicate that the MAPK pathway is a fundamental component of LTM formation in invertebrates and vertebrates. Thus, the MAPK cascade joins the cAMP/PKA pathway and the CREB transcriptional pathway as an evolutionarily conserved regulator of LTM consolidation (Figure 1). Work showing that MAPK is a major activator of plasticity-associated CREB-dependent gene expression also strongly suggests that MAPK signaling facilitates memory consolidation and L-LTP by promoting de novo CREB-regulated gene expression. There are a number of unanswered questions regarding the role of MAPK in neuronal plasticity and memory formation. Is CREB a target of Ras/MAPK signaling during memory consolidation? How is MAPK activated during adaptive neuronal plasticity and memory consolidation? What are the cytosolic and nuclear targets of MAPK that facilitate memory formation and modulate synaptic efficacy? Additional research using temporally and spatially restricted transgenic technologies should help clarify and confirm the role of Ras/MAPK signaling in LTM.

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NINDS Chronic Pain Information Page

Synonym(s): Pain - Chronic
Reviewed 07-01-2001

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What is Chronic Pain?
While acute pain is a normal sensation triggered in the nervous system to alert you to possible injury and the need to take care of yourself, chronic pain is different. Chronic pain persists. Pain signals keep firing in the nervous system for weeks, months, even years. There may have been an initial mishap -- sprained back, serious infection, or there may be an ongoing cause of pain -- arthritis, cancer, ear infection, but some people suffer chronic pain in the absence of any past injury or evidence of body damage. Many chronic pain conditions affect older adults. Common chronic pain complaints include headache, low back pain, cancer pain, arthritis pain, neurogenic pain (pain resulting from damage to the peripheral nerves or to the central nervous system itself), psychogenic pain (pain not due to past disease or injury or any visible sign of damage inside or outside the nervous system).

Is there any treatment?
Medications, acupuncture, local electrical stimulation, and brain stimulation, as well as surgery, are some treatments for chronic pain. Some physicians use placebos, which in some cases has resulted in a lessening or elimination of pain. Psychotherapy, relaxation and medication therapies, biofeedback, and behavior modification may also be employed to treat chronic pain.

What is the prognosis?
Many people with chronic pain can be helped if they understand all the causes of pain and the many and varied steps that can be taken to undo what chronic pain has done. Scientists believe that advances in neuroscience will lead to more and better treatments for chronic pain in the years to come.

What research is being done?
Clinical investigators have tested chronic pain patients and found that they often have lower-than-normal levels of endorphins in their spinal fluid. Investigations of acupuncture include wiring the needles to stimulate nerve endings electrically (electroacupuncture), which some researchers believe activates endorphin systems. Other experiments with acupuncture have shown that there are higher levels of endorphins in cerebrospinal fluid following acupuncture. Investigators are studying the effect of stress on the experience of chronic pain. Chemists are synthesizing new analgesics and discovering painkilling virtues in drugs not normally prescribed for pain.

Select this link to view a list of all studies currently seeking patients.

Organizations

American Chronic Pain Association (ACPA)
P.O. Box 850
Rocklin, CA 95677-0850
ACPA@pacbell.net
http://www.theacpa.org
Tel: 916-632-0922 800-533-3231
Fax: 916-632-3208

American Council for Headache Education

19 Mantua Road
Mt. Royal, NJ 08061
achehq@talley.com
http://www.achenet.org
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National Headache Foundation
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Tel: 773-388-6399 888-NHF-5552 (643-5552)
Fax: 773-525-7357

National Foundation for the Treatment of Pain
P.O. Box 70045
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Mayday Fund [For Pain Research]
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136 West 21st Street, 6th Floor
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Developments in mitogen-induced extracellular kinase 1 inhibitors and their use in the treatment of disease

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Multiple signal transduction pathways converge on the Raf-mitogen-induced extracellular kinase (MEK)-extracellular signal-regulated kinase (Erk) cascade to effect diverse cellular processes, including proliferation, differentiation, survival, apoptosis and organ functions such as memory consolidation. Improper activation of this pathway contributes significantly to numerous diseases, including cancer and various immune disorders. Specific inhibition of this signalling cascade thus offers great therapeutic potential for many diseases. Since the discovery of the first MEK1 inhibitor in 1995, several novel classes of inhibitors, with varying selectivity for MEK1, have been developed. Clinical applications for some of these have been investigated, with the majority focusing on proliferative diseases in which abnormally increased Erk activity plays a major role, most notably cancer, or immunological and inflammatory conditions such as arthritis and organ transplant rejection. To a lesser extent, ischaemia/reperfusion (I/R) injury and chronic pain disorders have also been targeted.

Keywords: inflammation, inhibitor, mitogen-activated protein kinase (MAPK), mitogen-induced extracellular kinase 1 (MEK1), proliferative disorders

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1. Introduction

Extracellular signal-regulated kinase (Erk) [1] belongs to the mitogen-activated protein kinase (MAPK) family and is highly conserved among eukaryotes. This pathway consists of the sequential activation of MAPK kinase kinase (Raf), the mitogen-induced extracellular kinase, MEK1/2 (MAPK/Erk kinase) and Erk1/2 [2]. Ras-induced activation of Raf leads to activation of MEK1 by phosphorylation of two serine residues, Ser218 and Ser222 [3]. MEK1, a dual specificity protein kinase, then activates Erk1 and Erk2 through phosphorylation on residues Thr183 and Tyr185 at a Thr-Glu-Tyr (TEY) site. In turn, Erk phosphorylates and activates nuclear transcription factors, such as ternary complex factor (TCF)/Elk-1, which regulate immediate-early genes, including the AP-1 components *fos* and *jun*. Erk also phosphorylates on the serine or threonine, followed by a proline (S/T-P motif) of substrates in other cellular compartments [4].

Multiple signal transduction pathways converge on the Raf-MEK-Erk cascade to effect diverse cellular processes including proliferation, differentiation, survival, response to DNA damage or apoptosis [5-9] and organ functions such as memory consolidation

[10]. The most well characterised activation of this cascade is mediated by receptor tyrosine kinases, such as growth factor receptors, through the small G protein, Ras [4]. Erk activation by growth factors leads to upregulation of D-type cyclin, thereby activating the cyclin-dependent kinases, Cdk4/6 [11-13]. Cdk4/6-cyclin D activity phosphorylates retinoblastoma (Rb) protein, resulting in the release of E2F1, which subsequently induces the transcription of genes essential for cell cycle progression from G1 into S phase [14]. A large body of evidence indicates that inappropriate activation of the Erk cascade contributes to tumorigenesis. Murine studies have revealed that activation of Erk is responsible for Ras-induced cell transformation. Inhibition of Erk activity blocks the growth of Ras-transformed cells [15-17] and constitutive activation of Erk is sufficient to induce cellular transformation [18]. In contrast to murine tumour models, activation of RalGEF, but not Raf, is sufficient for Ras transformation of primary human embryonic (HEK) epithelial cells ectopically expressing human telomerase (hTERT) and SV40 T-Ag genes (HEK-HT) [19]. Although Ras12V37G in HEK-HT cells (which activates only RalGEF) produced ~ 30% of the number of soft agar colonies, compared to those induced by Ras12V, concomitant expression of Ras12V37G and Ras12V35S, which activates only Raf, resulted in greater HEK-HT-cell growth in soft agar than cells expressing Ras12V [19]. Furthermore, ectopically expressed Ras12V37G in HEK-HT cells is insufficient to allow tumour formation in athymic mice [19]. Thus, activation of the Raf/MEK/Erk pathway is required for effective tumorigenesis in humans. This is consistent with observations that a large proportion of human cancers display elevated Erk activity. Increased Ras activity is found in > 30% of human cancers [20,21], and elevated Erk activity is found in 36% of human cancer cell lines and primary tumours [22]. Very recently, mutations in B-Raf, which increase its activity, were found in > 66% of melanomas, and expression of such Raf mutants in NIH3T3 cells led to transformation [23]. MEK1 itself is also overexpressed in a number of primary human tumours [22]. Furthermore, whilst increased Erk activity plays a key role in cell cycle progression, it is also important in other aspects of tumorigenesis, such as angiogenesis and metastasis. Deletion of MEK1 has been found to significantly attenuate angiogenesis [24]. Activation of Erk by the hepatocyte growth factor (HGF) contributes to HGF-induced epithelial cell dispersion [25-28], thus promoting tumour invasion. In aggregate, the multiple functions of the Raf-MEK-Erk pathway in promoting cell proliferation, angiogenesis, and tumour metastasis make inhibition of this pathway by specific inhibitors a very attractive target for cancer therapy.

Although dysregulated activation of the Erk cascade in many cancers is well-established, evidence for Erk involvement in inflammatory and immune responses, in conditions as diverse as arthritis, glomerulonephritis, asthma and organ transplantation, has emerged only recently [29-32]. Knocking out the serine/threonine protein kinase Tpl2/Cot was found to specifically inhibit lipopolysaccharide (LPS)-induced MEK1/Erk activation and thus TNFα and cyclooxygenase 2 (COX2) production in macrophages [33,34]. Consistent with the high levels of Raf, MEK and Erk expressed in the CNS, Erk activation through the Rap1/B-Raf/MEK pathway in response to Ca^{2+} and cAMP has also been found to play an essential role in neuronal plasticity and memory formation [6]. Inhibitors that target this signalling pathway may therefore be potentially useful in a wide spectrum of clinical conditions.

Theoretically, any of the three primary kinases in the Raf-MEK-Erk cascade could be targeted for clinical application. However, recent developments strongly suggest that MEK1 is not the only substrate for Raf. Whilst MEK/Erk activation is not compromised in Raf-1 deficient mice (c-Raf1$^{-/-}$), this mutation is lethal to the embryo, with increased apoptosis in liver and other embryonic tissues, indicating that Raf has anti-apoptotic functions that are independent of MEK/Erk [35,36]. MEK1/2 are highly specific Erk-activating kinases and no other substrates have been identified [37,38]. Although MEK1 and MEK2 share 90% amino acid sequence identity, inhibition of MEK1 abrogates Erk activation in nearly all cells examined, suggesting that MEK1 is the dominant Erk-activating kinase [39].

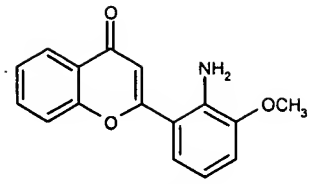
The development of MEK1 inhibitors is a relatively recent advance, with the first selective inhibitor, PD98059 (compound 1), being described in 1995 [15]. This inhibitor has proven indispensable in investigations of the role of Erk activation in diverse cellular processes, which is reflected by the large number (2671) of references making use of PD98059 in the National Library of Medicine database. Several classes of novel compounds that function as MEK inhibitors, and increasing numbers of clinical applications for these, are being described and patented and these are reviewed herein.

2. Small-molecule mitogen-induced extracellular kinase 1 inhibitors

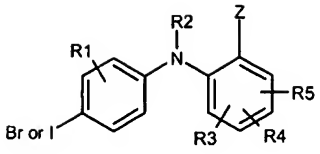
MEK1 inhibitors can be classified as either small molecule compounds or antisense inhibitors.

2.1 PD98059 and U0126

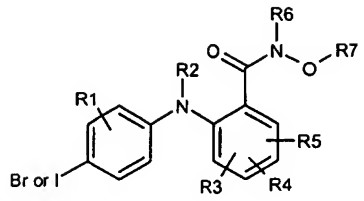
The two most widely used MEK1 inhibitors are PD98059 (compound 1) and U0126 (compound 2). PD98059, an aminoflavone of the formula 2-(2-amino-3-methoxyphenyl)-4-oxo-4H-[1]benzopyran, was first described in 1995 [15] and patented by Warner-Lambert, Inc. (Parke-Davis, now Pfizer) in 1996 [201,202]. U0126 has the formula 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene [40]. Both PD98059 (1) and U0126 (2) contain two benzyl rings separated by different molecules. They are not competitive with ATP and function as MEK inhibitors through a similar mechanism [41]. Consistent with their similar chemical structures, PD98059 and U0126 compete with one another for MEK1 binding, indicating that they interact with MEK1 at the same, or an overlapping, site [40]. The affinity of U0126 for MEK1, however, is ~ 100-fold higher than that of PD98059, corresponding to its lower IC_{50} value of 0.072 μM, as compared



1 PD98059

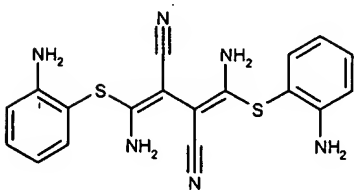


3a



3b

R1, R2, R3, R4, R5 and R6 = H or substituent groups such as alkyl
R7 = H or an organic radical
X = COOR7



2 U0126

with the IC_{50} value of 2 – 10 μM for PD98059, determined in an *in vitro* MEK1 protein kinase assay [40,42-44]. These MEK1 inhibitors also display some inhibition of MEK5 activation [45]. Compared to the inhibition of epidermal growth factor (EGF)-induced Erk activation, both compounds inhibit EGF-induced MEK5-Erk5 activation with much lower potency [46]. PD98059 was also found to inhibit COX1/2 with an IC_{50} value of 1 – 4μM, a potency similar to its inhibition of MEK1 activation [47].

PD98059 and U0126 were reported to suppress MEK1 activation by Raf, rather than to inhibit MEK1 activity. Phosphorylation on S217 and S221 is known to activate MEK1. Dephosphorylated MEK1 has very low kinase activity, which is increased 7000-fold following Raf-induced phosphorylation of S217 and S221. Substitution of both serine residues (S217 and S221) with glutamic acid mimics the phosphorylated state and increases kinase activity by 40-fold [48]. PD98059 could inhibit dephosphorylated MEK1 and $MEK1^{S217E/S221E}$ with an IC_{50} value of 2μM, but was unable to inhibit the kinase activities of either Raf-activated recombinant MEK1 or MEK1 immunoprecipitated (IP) from EGF-stimulated Swiss 3T3 cells at 100 μM concentrations [42]. This indicates that PD98059 interacts specifically with the inactive form of MEK1. It is consistent with the observations that, *in vitro*, PD98059 inhibits c-Raf- or MEKK1-initiated MEK1 phosphorylation and activation at an IC_{50} value of 4 μM [42]. In *in vitro* assays, U0126 at 10 μM does not inhibit MEK1 phosphorylation by Raf immunoprecipitated (IP) from phorbol 12-myristate 13-acetate (PMA)/PHA treated Jurket cells [40] However, it does inhibit recombinant active MEKK1 (MEKK-C)-mediated MEK1 phosphorylation with an IC_{50} value of 3 μM [49]. Phosphorylation of recombinant MEK1 by Raf IP from EGF-treated Swiss 3T3 cells is strongly suppressed by U0126 at an IC_{50} value of 0.5 μM [41]. It is not clear what might cause the differences in U0126 behaviour reported in [40] compared to that reported in [41,49]. However, the latter observations are consistent with the finding that U0126 competes with PD98059 for MEK1 interaction [40]. Like PD98059, U0126 inhibits MEK1 activation by Raf or MEKK-C with 50-fold greater potency than its inhibition of the kinase activity of activated MEK1 *in vitro* [41,49]. When MEK1 and Erk2 were IP from EGF-treated Swiss 3T3 cells, with or without U0126, it was found that MEK1 activity was suppressed five to tenfold more potently than Erk2 activity [41], supporting the notion that U0126, like PD98059, inhibits activation of MEK1 by Raf, instead of blocking MEK1 kinase activity. In an effort to address the effects of PD98059 and U0126 on MEK1 phosphorylation in cells, it was found that these inhibitors increase serum-stimulated immunoblot signals detected by an antibody specific for S217/S221-phosphorylated MEK1 [49]. Since MEK1 IP from both U0126 and EGF-treated cells displays reduced kinase activity [41], it is thus unclear if the antibody might recognise sites other than the two phosphorylated residues. Taken together, evidence supports that PD98059 and U0126 inhibit MEK1 activation by Raf with much greater potency than they inhibit MEK1 activity.

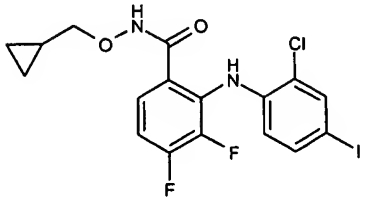
2.2 Benzoic acid derivatives and benzhydroxamic acid derivatives

A group of small-molecule MEK inhibitors with chemical structures similar to those of PD98059 and U0126 were patented from 1999 – 2002 by Barrett *et al.* of Warner-Lambert Co. (Pfizer) [203-205]. Three similar patents [203-205] described 2-(4-bromo or 4-iodo phenylamino) benzoic acid derivatives with different substitutive groups (compound 3a). The same team also patented small-molecule MEK1 inhibitors with the formula 4-bromo or 4-iodo benzhydroxamic acid derivatives [206] (compound 3b). Like PD98059 and U0126, molecules of both groups inhibit MEK1 activation *in vitro* and *in vivo* but do not compete with ATP [203,206]. These inventions also demonstrated that, although both benzoic acid and benzhydroxamic acid derivatives inhibit insulin-induced Erk activation with an IC_{50} value of 3 μM, they do not inhibit insulin-induced glucose

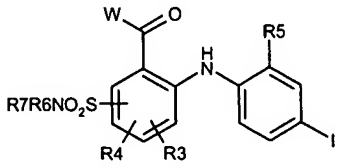
uptake in 3T3-L1 adipocytes at concentrations as high as 10 μM [203,206]. This demonstrates their specificity in inhibiting mitotic, but not metabolic, cellular processes. This important property underlies the finding that these inhibitors could reverse the transformed phenotype of some cell types, as measured by growth in soft agar, while having relatively low toxicity [203,206]. For therapeutic purposes, both groups of inhibitors could be administered to animals at doses up to 500 mg/kg body weight, either parenterally or orally [203,206].

One of the 4-iodo benzhydroxamic acid derivatives, identified as PD184352 (compound 4), with the formula 2-(2-chloro-4-iodo-phenylamino)-N-cyclopropylmethoxy-3,4-difluorobenzamide, has been characterised in detail [206]. The reason for choosing this compound (structure number 95 of 102 identified in the patent) for further characterisation was not given. Although PD184352 (4) is a highly potent inhibitor of MEK1 activation (IC_{50} = 1 nM), several other structures (listed in the patent as numbers 1, 37, 84, 89, 101, 102, 37) have comparable, or even higher (structure number 87), potency as MEK1 inhibitors [206]. In mice subcutaneously implanted with the colon tumour cell line C26/clone10, followed, after 1 day, by the administration of PD184352 (4) either intraperitoneally at 200 mg/kg or orally at 300 mg/kg body weight, tumorigenesis was inhibited by 100% and 83%, respectively. The effect on tumour regression was not determined. At such doses, PD184352 was not associated with weight loss over the 15 day experimental period [206]. This high efficacy in preventing tumour progression with no detectable toxicity is very appealing when considering the high level of toxicity of drugs currently used in cancer therapy. Clinical applications for PD184352, and several other specific compounds of this class, will be discussed in subsequent sections.

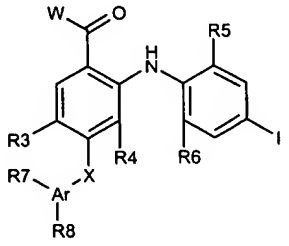
Similar to PD98059 (1) and U0126 (2), PD184352 (4) also suppresses Erk5 activation with much lower potency than it does MEK1 activation [50]. PD184352 inhibits serum-stimulated Erk1 activation with an IC_{50} value of < 1 μM, but does not inhibit serum-stimulated Erk5 activation at 20 μM [50]. Doses of PD184352, which induce G1 cell cycle arrest through inhibition of cyclin D expression, Erk1 but not Erk5 activation is suppressed. This suggests that PD184352 inhibits cell proliferation by abolishing Erk activation [50].



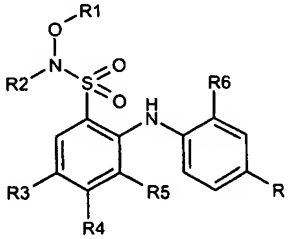
4 PD184352



5a W = OR1, NR2OR1, NR_AR_B, NR_2NRARB, $NR2(CH_2)_{2-4}NRARB$
R1, R2, RA, R6, R7 = H, phenyl and substituent groups
RB = H or substituent groups
R3 = H, F, Cl, Br or NO_2
R4 = H or F
R5 = H, CH_3 or Cl



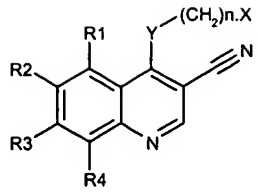
5b W = OR1, NR2OR1, NRARB, NR2NRARB, $NR2(CH_2)_{2-4}NRARB$
R1, R2, RA = H, phenyl and substituent groups
RB = H or substituent groups
R3 = halo, NO_2 or other substituent groups
R4 = H or F
R5, R6 = H, CH_3, halo or NO_2
R7, R8 = H, halo or other substituent groups
Ar = phenyl or pyridyl (2- or 3- or 4- pyridyl)
When Ar = a pyridyl, R7, R8 = H
X = O, S or NH



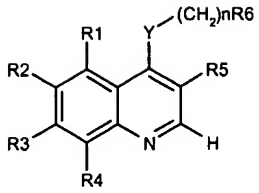
5c R1 and R2 = H or substituent groups (i.e., alkyl)
R3 and R4 = H, F, NO_2, Br or Cl
R5 = H or F
R6 = H, F, Cl or CH_3

2.3 Benzenesulfonamide, sulphohydroxamic acid and diarylamine derivatives

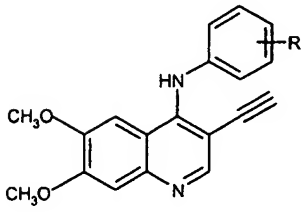
The benzenesulfonamide derivatives (such as compound 5a) and the 4-arylamino, 4-aryloxy, and 4-arylthio diarylamines and derivatives, such as compound 5b, were patented by Barrett *et al.* of Warner-Lambert Co. (Pfizer) in 2000 [207,208]. The same company also patented sulphohydroxamic acids and sulphohydroxamates in 2000, with the structure of compound 5c [209]. These compounds inhibit MEK1 activation without inhibiting the MAP kinase kinase, MKK3, protein kinase C (PKC), Cdk2A, phosphorylase kinase, EGF receptor (EGFR), platelet-derived growth factor receptor (PDGFR) or c-Src [207-209]. The IC_{50} value, as determined in an *in vitro*



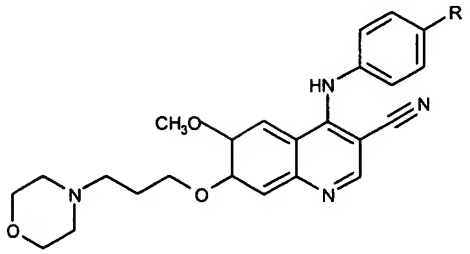
6a X = a cycloalkyl
Y = -NH-, -O-, -S- or -NR-
R1, R2, R3 and R4 = H or a sustituent group



6b Y = -NH-, -O-, -S- or NR7
R1, R2, R3 and R4 = H or a substituent group
R5 = F, Cl, Br or CN
R6 = R8-X-R9
R7 is an alkyl
R8 = divalent cycloalkyl or pyridinyl, pyrimidinyl or phenyl
R9 = (CH_2)mR10
(where m = 0 – 3 and R10 = aryl, cycloalkyl or heterpocyclic ring)



6c



6d

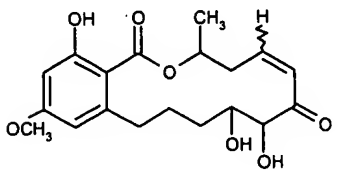
MEK1 protein kinase assay, was in the micro/submicromolar range for benzenesulfonamide derivatives, 0.04 μM for PD195928 (a diarylamine derivative), and 0.965 μM for PD0297447 (a sulphohydroxamic acid/sulphohydroxamate) [207-209]. All of these compounds were claimed to have clinical potential in treating a variety of proliferative diseases including cancer, restenosis, psoriasis and autoimmune disease and other uses such as in organ transplantation. However, no supporting data were provided [207-209]. In general, the potency of these inhibitors in blocking MEK1 activity, is rather low.

2.4 Quinoline derivatives

A group of quinoline derivatives have been patented as MEK inhibitors [210-213]. Although the 3-cyano quinoline derivatives, such as compound 6a described by the American Cyanamid Co. in 1998, display MEK inhibition, they are actually much more potent inhibitors of a number of growth factor receptor protein tyrosine kinases including EGFR, epithelial cell kinase (ECK) and vascular endothelial growth factor receptor (VEGFR) [210]. Some derivatives of this class blocked the protein tyrosine kinase activities of EGFR and ECK with an IC_{50} value of 0.021 nM and 1 nM, respectively, as compared to the lowest IC_{50} value of 0.2 μM for MEK inhibition [210]. These compounds are thus better inhibitors for a number of receptor protein tyrosine kinases than for MEK1. AstraZeneca has subsequently patented similar quinoline derivatives with a cyanide, fluoride, chloride or bromide group in the 3 position (compound 6b) [211-213]. The most potent compounds inhibit MEK1 activity with an IC_{50} value between 0.15 and 1.5 μM in an *in vitro* MEK1 assay; the IC_{50} values for other, less potent, compounds is typically < 20 – 30 μM [211-213]. The quinoline derivatives with the formulas shown in compounds 6c (3-cyano-4-(phenoxyanilino)-6,7-dialkoxyquinolines) and 6d (4-anilino-3-cyano-6-methoxy-7-(3-morpholino-4-yl-proxy) quinolines), might be potent MEK inhibitors. Substitution of R (compound 6c), with either a para-phenoxy or para-benzyl, produced compounds that inhibit MEK1 activation with IC_{50} values of 6.2 and 3.6 nM, respectively, as determined in an *in vitro* coupled MEK assay [51]. A flexible linker between the two phenyl rings in position 4 (compound 6c) is critical for potent MEK1 inhibition [51]. Substitution of R in compound 6d with a phenoxy, benzyl or phenylsulfonyl also generated potent MEK inhibitors with IC_{50} values of 2.4, 2.7, and 1.1 nM, respectively [51]. These compounds block growth of human colon tumour LoVo cells (IC_{50} = < 1 μM) [51]. Although compounds with the formulae of 6c or 6d do not inhibit Cdk2, Cdk4, Akt, Erk, EGFR, HER2/NEU, ECK or VEGF-related KDR (VEGFR-2), whether they might inhibit other MKKs is not known. Since these compounds are competitive with ATP and the ATP concentrations used in the *in vitro* assays were not given [51], it is unclear if their potency as MEK1 inhibitors is greater than those reported by AstraZeneca.

2.5 Resorcylic acid lactones

The resorcylic acid lactones have been isolated from microbial extracts by two groups. Compounds of the general formula (compound 7) and its isomers, such as compounds 8 and 9, were purified by a Merck research group [214]. These were derived from a fungus of the genus *Phoma*, deposited with the American Type Culture Collection (ATCC) under the designation 74403. A representative compound, L-783,277, has the formula [5Z]-3,4,9,10,11,12-hexahydro-8,9,16-trihydroxy-14-methoxy-3-methyl-1H-2-benzoxacyclotetradecin-1,7(8H)-



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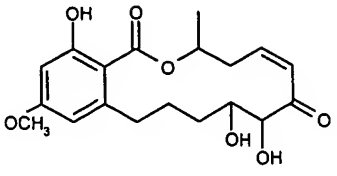

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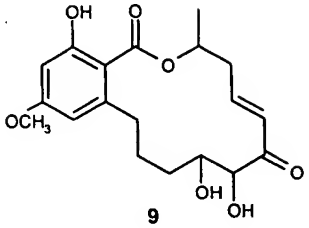

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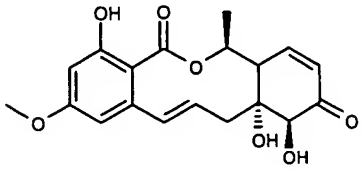


10 Ro 09-2210

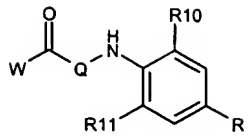
dione (compound 8). Unlike earlier inhibitors, it is competitive with ATP. Hence, the IC_{50} value is dependent on the assay ATP concentration used. In the presence of 10 μM ATP, L-783,277 inhibits MEK1 with an IC_{50} value of 4 nM [43,52,214]. L-783,277 is also a weak inhibitor of Lck, a protein tyrosine kinase, but is unable to inhibit other protein kinases screened including Raf, PKA and PKC [52]. Although it was indicated in the patent that a compound of structure 8, such as L-783,277, could be used to treat cancer, no data are available to support this claim [214]. Another stereoisomer, Ro 09-2210 (compound 10) was isolated from the mycelial cake of *Curvularia* sp. by Williams *et al.* from Roche [53]. Ro 09-2210 has no inhibitory activity against a range of Ser/Thr- and Tyr-specific protein kinases (IC_{50} > 100 μM). It inhibits MEK1 *in vitro* with an IC_{50} value of 59 nM [53].

2.6 Other small-molecule mitogen-induced extracellular kinase 1 inhibitors

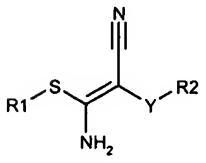
Benzoheterocycles of the general formula shown in compound 11a were patented by Barrett *et al.* of Warner-Lambert Co. (Pfizer) in 2000 [215]. These compounds inhibit MEK1/2, but not MKK3, PKC, Cdk2A, phosphorylase kinase, EGFR, PDGFR or c-Src [215]. The derivatives specified as PD205293, 7-fluoro-6-(4-iodo-2-methyl-phenylamino)-1H-benzoimidazole-5-carboxylic acid, and PD254552, 7-fluoro-6-(4-iodo-2methyl-phenylamino)-1H-benzoimidazole-5-carboxylic acid cyclopropylmethoxy-amide, inhibit MEK1 with an IC_{50} value of 14 nM and <10 nM, respectively [215]. These compounds were stated to be tested in multiple disease models including tumourigenesis, murine collagen-induced arthritis, murine Streptococcal cell wall (SCW)-induced monoarticular arthritis, murine ear-heart transplantation and murine ovalbumin-induced eosinophilia. However, no data were provided in the patent [215].

Amino-thio-acrylonitriles with two formulae (shown as compound 11b) have also been patented as MEK inhibitors [216]. Formula I (11b) is actually closely related to the chemical structure of U0124 (compound 11c), a product generated in the process of synthesising U0126. Indeed, substitution of R1 and Y-R2 in formula I with specific groups would yield U0126 (compound 2). Both U0126 and the amino-thio-acrylonitriles were patented by the same research team [40,216]. The ability of the latter in inhibiting MEK1 is not known. However, the characteristic feature of all potent MEK1 inhibitors, including PD98059, U0126 and those discussed in Sections 2.2 and 2.3 (compounds 3a and b), is the presence of a benzyl-bearing group at positions R1 and R2. Based on this, and on a report showing that U0124 does not inhibit MEK1 [40], it might be possible to predict that other substitutions in these locations would not generate good MEK1 inhibitors.

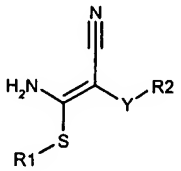
DuPont has recently published data on a new MEK inhibitor, SL327, the structure of which is similar to U0126 [54]. Determined by an *in vitro* kinase assay, the IC_{50} values for U0126 and SL327, for MEK1/2 inhibition, are 0.07/0.06 μM and 0.18/0.22 μM, respectively [55]. SL327 does not inhibit other kinases tested including Erk1, MKK3, MKK4, c-JUN N-terminal protein kinase (JNK), PKC, PKA, and CamKII [55,56]. Based on the structural homology with U0126, SL327 may be non-competitive with ATP, although specific information regarding this is, as yet, unavailable. Assayed in COS7 cells [40], U0126 and SL327 both suppress PMA-induced AP-1 promoter reporter activity with an IC_{50} value of 0.96 μM and 2.03 μM, respectively [55]. Further information concerning the *in vivo* specificity of SL327 is needed, however, given the observation that SL327 is more effective than U0126 in inhibiting uterine Erk activation induced by sesame oil infusion into the uterine horn, despite the greater *in vitro* MEK inhibition shown by U0126 [55]. In the 13 publications which used SL327 [54-66], 10 injected the compound intraperitoneally to block neuronal Erk activation. Whether this reflects unique pharmacological properties of SL327, which enable it to pass through the blood–brain barrier, is not certain [56]. Due to the very limited information available about SL327 and its homology to U0126, a direct comparison of their *in vitro* and clinical effects should be made.



11a W = OH or a carboxylic acid derivative
Q = heterocyclo-condensed ortho-phenylene residue
R10 and R11 = H, CH_3, halo or NO_2

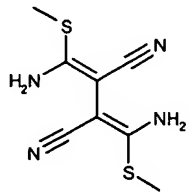


Formula I



Formula II

11b Y and R1 = phenyl or substituent group
R2 = H or a substituent group



11c U0124

Hymenialdisine, isolated from the marine sponge *Hymeniacidon* sp., inhibited MEK1-mediated Erk activation with an IC_{50} value of 6 – 9 nM, determined by an *in vitro* enzyme-linked immunosorbent assay (ELISA)-based kinase assay [67]. Hymenialdisine also inhibited the growth of human colon cancer LoVo and Caco-2 cells with IC_{50} values of 4 – 8 µM and 0.6 – 0.7 µM, respectively [67]. The effect of hymenialdisine on other kinases was not determined [67]. However, other investigators have found that hymenialdisine potently inhibited Cdks, GSK-3, and CK1 at nanomolar concentrations *in vitro*, but poorly inhibited MEK activity [68]. Hymenialdisine was also found to inhibit Chk1 and Chk2 kinases *in vivo* at micromolar concentrations [69]. Thus, hymenialdisine might not be a specific MEK inhibitor.

Since phosphorylation of Erk by MEK is facilitated by MEK binding through its N-terminal docking site [70], destruction of this interaction would lead to inhibition of Erk activation. The synthetic peptide (amino acids 1 – 20 of MEK2) encompassing this docking site was found to inhibit Erk2 phosphorylation by MEK1 *in vitro* with an IC_{50} value of 25 µM [70]. The specificity and effectiveness of this approach *in vivo* is yet to be determined. Alternatively, the docking sites of MEK1/2 could be cleaved by anthrax lethal factor (LF) between residues Pro8 and Ile9 and between residues Pro10 and Arg11 in MEK1 and MEK2, respectively [71,72]. This cleavage prevents Erk from being activated by MEK1/2 and thereby inhibits the growth of Ras12V-transformed NIH3T3 cells in soft agar and in athymic nude mice [73]. However, the action of LF might not be specific to MEK1, as it also cleaves MKK3 [74].

2.7 Antisense inhibitors

ISIS Pharmaceuticals, Inc. patented antisense inhibitors of MEK1 expression [217,218]. A series of antisense oligodeoxynucleotides matched to MEK1 mRNA were synthesised and tested for efficacy to down-regulate MEK1 mRNA. One phosphorothioate oligodeoxynucleotide, which targets at 911 in the coding region (Genbank accession no. L11284), inhibited MEK1 mRNA transcript abundance by 87% as measured by quantitative real-time polymerase chain reaction (PCR). This was the maximal inhibition achieved among all antisense inhibitors tested [217]. In human leukaemia U937 cells, vector-based expression of full-length MEK1 antisense cDNA (1.3 kb) suppressed bufalin-induced AP-1 transcriptional activity [75]. Antisense oligonucleotides targeting other molecules have been safely administered to humans [76].

3. Mitogen-induced extracellular kinase 1 inhibitors and their clinical application

Increasing numbers of patents addressing the clinical application of MEK1 inhibitors to specific diseases have emerged over the last several years. The majority deal with proliferative diseases such as cancer, or immunological and inflammatory conditions, including arthritis and organ transplant rejection. To a lesser extent, ischaemia/reperfusion (I/R) injury and chronic pain disorders have also been targeted.

3.1 Mitogen-induced extracellular kinase 1 inhibitors in the treatment of cancer and other proliferative disorders

A large body of evidence supports an essential role of Erk activation in tumorigenesis [15-18,23]. Targeting components of the Erk activation pathway thus represents a rational potential therapy for these disorders. Two strategies are currently being tested in the treatment of human tumours implanted in mice, either by the use of MEK1 inhibitors alone or MEK1 inhibitors in combination with conventional chemotherapeutic agents to potentiate therapy.

3.1.1 Inhibitors of mitogen-induced extracellular kinase 1 and the prevention of tumour growth

Human colon cancer and melanoma display a high level of Erk activity and are sensitive to MEK1 inhibitors, suggesting that Erk activation is essential in sustaining the growth of these cancers. Although PD184352 inhibited the growth of three colon tumour cell lines (C26, HT-29, and colo205) with high levels of Erk activity (IC_{50} = 0.12 – 0.18 µM), clonogenic assays revealed that PD184352 at 10 µM did not affect the growth of

MCF-7 (breast cancer) and PC3 (prostate cancer) cells, both of which have low levels of Erk activity [77]. Furthermore, PD98059, U0126 and PD184352 were found to effect only G1 cell cycle arrest in normal melanocytes, but to induce both G1 cell cycle arrest and apoptosis in melanoma cells [219]. PD184352 caused apoptosis in 24.5% of melanoma cells at 1 μM and 71% at 20 μM after 72 h. Erk activity in melanoma cells is higher than in normal melanocytes. Inhibition of MEK1 activation also prevents tumour metastasis. PD184352 blocked HGF-induced HT-29 cell scattering and the invasion of colon 26 cells through Matrigel membranes [77]. Both U0126 and PD98059 also dose-dependently inhibited the invasion of A375 melanoma cells [219], which is at least partially due to decreased matrix metalloprotease-9 (MMP9) and urokinase plasminogen activator (UPA) expression [78]. Administration of MEK1 inhibitors, either intravenously or locally, into tumours, significantly decreased tumour size in M14-MEL xenografts in athymic nude mice [219].

The patent by the Van Andel Institute on the treatment of melanoma wih MEK inhibitors, published in March 2002, also presents patient data [219]. One hundred and fifty patients who failed conventional therapy in histologically diagnosed metastatic melanoma were given either an MEK1 protease or a small-molecule MEK1 inhibitor intravenously for 12 treatments, specific compounds used were not stated. In 66% of patients there was a > 50% reduction in tumour diameter with no new lesions. About 10% of patients suffered adverse effects including gastrointestinal, allergic and systemic symptoms [219]. It was not clear if the side effects were due to an MEK1 protease or a small-molecule inhibitor [219]. However, it was reported that oral administration of PD184352 for 2 weeks, with cumulative dosing up to 6 g/kg body weight, did not affect weight gain in mice [77], indicating that, at least in mice, this compound is likely to be unaffected by significant toxicity.

It is also worth noting that although PD184352 administered orally at 200 mg/kg body weight at day 1 after colon tumour C26/clone 10 implantation could block 80% of tumour growth [77,206], oral administration of the compound from doses of 48 – 200 mg/kg body weight inhibited growth of formed tumours by 59 – 69% [77]. The mechanism underlying this observation may be due to a similar property shared by PD184352, PD98059 and U0126, namely that these compounds were suggested to suppress MEK1 activation, rather than inhibit MEK1 activity *in vivo* [41]. This suggests that for efficient inhibition of MEK1 in cultured cells, the basal level of MEK1 activation needs to first be reduced by serum starvation. For cancer therapy, however, a rational approach would be the combination of PD98059, U0126, or PD184352 with other compounds, which reduce the basal level of MEK1 activity.

3.1.2 Combination therapy

3.1.2.1 Combination of Ras inhibition with a mitogen-induced extracellular kinase 1 inhibitor, PD98059

Merck & Co. has described combination therapy consisting of a farnesyl protein transferase inhibitor (FTI), which interferes with Ras activation, together with an MEK1 inhibitor [220]. Studies were described for PD98059, but no data were presented in the patent. Since Ras also activates pathways other than those downstream of MEK1/2 to facilitate tumourigenesis, the addition of MEK1 inhibitors may enhance the tumour inhibitory effects of an FTI. Indeed, exposure of Ras-transformed Rat2 fibroblasts to MEK1 inhibitors increased their apoptotic response to an FTI. Levels of phosphorylated Erk were suppressed to a greater degree in the presence of both agents when compared to either agent alone [79]. Glass *et al.* have also shown that treatment of glioblastoma cell lines, with an FTI, attenuated cell growth in soft agar and induced G2 arrest. This was associated with decreased phosphorylated Erk levels with no change in the total Erk level [80].

3.1.2.2 Combination of paclitaxel (Taxol™, Bristol-Myers Squibb) with PD184352 in cancer therapy

Pfizer has patented the use of mitotic inhibitors such as paclitaxel (Taxol™, Bristol Myers Squibb) together with MEK1 inhibitors, particularly the benzhydroxamic acid derivatives [221]. Several compounds in the group were tested in combination with paclitaxel, most notably PD184352 [221]. Treatment of colon 26 carcinoma cells with 100 nM paclitaxel for 48 h, followed by 30 nM PD184352 for an additional 48 h induced apoptosis in 44% of cells compared with 18% with paclitaxel alone and 5% with the MEK1 inhibitor alone [221]. Similar results were also obtained in HT-29 cells [221]. However, it has also been reported that inhibition of MEK1 activation prior to administration of paclitaxel actually reduced cell death in U937 cells [81]. Combination of paclitaxel at 10 nM with 100 nM PD184352 only very marginally enhanced paclitaxel-induced apoptosis from 41 to 47% in A549 (non-small cell lung carcinoma) cells [221]. Further complicating this issue, is that paclitaxel actually induced Erk activation, although addition of either PD98059 or U0126 did increase apoptosis about threefold [82]. The effectiveness of this combination in cancer therapy is thus not clear, although it is likely that effects are dependent on the type of cancer.

3.1.2.3 Combination of a cell cycle checkpoint abrogation agent, UCN-01 with PD184352

In 2002, Virginia Commonwealth University patented the combination of a cell cycle checkpoint-abrogating agent, such as 7-hydroxystaurosporine (UCN-01), with either an MEK1 or a PI-3K inhibitor for cancer therapy [222]. Addition of UCN-01 to either PD98059, U0126 or PD184352 synergistically enhanced apoptosis in several leukaemia cell lines as well as breast and prostate cancer cells [84,222]. Furthermore, this combination of agents reduced soft agar colony growth to 10% of control values and enhanced the radiosensitivity of tumour cells in clonogenic survival assays to low-dose (2 Gy) radiation. Importantly, there was no increase in apoptosis in normal haematopoietic cells or other primary cells [222]. UCN-01 was found to activate Erk by 2 h and this was accompanied by cell cycle arrest, suggesting that Erk activity might play a role in UCN-01-induced cell cycle arrest. This is consistent with the findings that Erk activity plays a role in $p21^{CIP1}$ expression and

in DNA damage-induced cell cycle arrest [8]. An MEK1 inhibitor reversed UCN-01-induced Erk activation, reduced the proportion of cells in the G2/M phase of the cell cycle and increased the number of apoptotic cells [222]. It is important to note that UCN-01 not only inhibits CHK1, but also blocks several other protein kinase activities with equal potency, including MAPKAP-K1b, MSK1, PKCα, PDK1, AMP-activated protein kinase (AMPK), phosphorylase kinase (PHK), and lymphocyte kinase (LCK) [41]. It is thus unclear if the synergistic effect of UCN-01 with PD98059 is due to its effect on cell cycle checkpoint abrogation.

3.2 Treatment of ischaemia with mitogen-induced extracellular kinase 1 inhibitors

The General Hospital Corporation in Boston, USA holds the only patent for the use of MEK1 inhibitors in the treatment of ischaemia [223]. This was applied specifically to a murine stroke model induced by temporary (1 – 2 h) middle cerebral artery occlusion (MCAO) followed by 3 min of reperfusion. In this model, Erk phosphorylation was increased in the cerebral cortex by I/R. PD98059 not only decreased phosphorylated Erk, but when administered into the cerebral ventricle 30 min prior to induction of ischaemia, it dose-dependently decreased infarct volume by 23% and 42% at 22 h after reperfusion using 50 and 10 μM, respectively of an MEK1 inhibitor [223]. This was associated with a reduction in neurological deficits at 72 h. Importantly, when U0126 was administered intravenously 10 min prior to reperfusion, following 3 h of focal ischaemia, infarct volume was decreased by 40% at 24 h, an effect that was sustained to 35 days with a 41% decrease in brain atrophy [84]. Pretreatment with U0126 had similar beneficial effects on infarct volume and neurological deficits when focal ischaemia was permanently induced. Protective effects were evident with treatment only up to 1 h following MCAO [84]. A second model of ischaemia was that associated with cardiac arrest, induced by bilateral carotid artery occlusion (BCAO) for 3.5 min. This was accompanied by decreased hippocampal phosphorylated Erk during ischaemia, which significantly increased soon after reperfusion. Again, intravenous U0126 given either before or up to 3 min after BCAO was protective [84].

3.3 Application of mitogen-induced extracellular kinase 1 inhibitors to immunological and inflammatory conditions

Erk activity is known to function in inflammatory and immune responses as cytokines, such as TNFα, IL-1β, LPS and chemotactic factors, such as C5a and IL-8 lead to Erk activation [31,85-87]. PD98059 blocked Erk activation and chemotaxis in neutrophils induced by IL-8 and C5a [87] and Erk activation by T cell receptor (TCR) ligation was inhibited by U0126 [32]. Inhibition of TCR engagement-induced Erk activation by U0126 impaired T cell activation and proliferation and decreased IL-2 synthesis [32]. Production by activated T cells of other cytokines such as TNFα, IL-3, IFN-γ, IL-6 and IL-10 was also decreased by PD98059 [88]. Consequently, a number of patents dealing with the use of MEK1 inhibitors in disorders in which the immune system plays a key role have recently emerged. These include inflammatory arthritis, asthma, organ transplant rejection, septic shock and viral infection. Interestingly, all of the applications discussed below have been patented by Warner-Lambert Co. (Pfizer). None have yet been studied in clinical trials.

3.3.1 Treatment and prevention of arthritis with mitogen-induced extracellular kinase inhibitors

Benzoic acid and benzhydroxamic acid derivatives [206] have been patented as potent antiarthritic agents [224]. PD184352, a benzhydroxamic acid derivative, was claimed to be effective in the treatment of the inflammatory disease, rheumatoid arthritis (RA), and the non-inflammatory degenerative joint disease, osteoarthritis (OA) [224]. Approximately 18 benzhydroxamic acid derivatives [206] were tested as antiarthritic agents and PD184352 was found to be the most potent [224]. In a monoarticular arthritis model, rats were injected with SCW antigen into an ankle joint. After 3 weeks, delayed-type hypersensitivity was induced with an intravenous injection of 100 μg SCW. PD184352 and a related compound, PD170611, administered 1 h prior to systemic injection dose-dependently inhibited paw swelling at 4 days. At 20, 60 and 200 mg/kg/day PD184352 inhibited swelling from day 4 to day 7 by 17, 30 and 53%, respectively. In animals treated with PD170611 at 300 mg/kg/day, paw swelling declined from 40% at day 4 to 0% at day 7 [224]. Surprisingly, a lower dose of 100 mg/kg/day led to slightly improved results, with a reduction in paw swelling of 48% [224].

MMP1 (collagenase) and MMP3 (stromelysin) play a crucial role in matrix degradation in rheumatoid arthritis [44,89]. Erk activation and stromelysin-1 production induced by the cytokine IL-1β in rabbit synovial fibroblasts were found to be inhibited by PD184352 [224]. In New Zealand White rabbits, PD184352, PD185625 and PD185848 also inhibited IL-1β induced cartilage degradation by 75, 63, and 43%, respectively [224]. PD184352 was also found to be therapeutically effective in a model of collagen-induced arthritis (CIA). CIA is a classic model for rheumatoid arthritis in which mice are immunised intradermally with collagen II and systemic inflammatory arthritis subsequently develops. Oral administration of PD184352 at 20, 60, and 200 mg/kg/day for 63 days reduced the incidence of arthritis to 60, 10, and 22%, respectively. The severity of arthritis correlated with the dose of PD184352 used, as disease scores decreased from 6.3 in control animals to 2.9, 0.6, and 0.333 in those treated with increasing doses of PD184352, as above [224]. While these results show that PD184352 may be a potent agent against inflammatory arthritis, they also imply that there may be a threshold dose for optimum therapeutic benefit. Finally, there is some evidence to suggest that MEK1 inhibitors may be a potential therapeutic target in OA. In cartilage explants from dogs in which OA had been induced by sectioning of

the anterior cruciate ligament, PD98059 decreased chondrocyte death [90].

3.3.2 Mitogen-induced extracellular kinase inhibitors in organ transplant rejection

Use of MEK inhibitors for the prevention and control of transplant rejection was patented in 2000 [225]. T cell responses, including proliferation and secretion of numerous cytokines, play a key role in the development and propagation of rejection. The cytokine IL-2 is the main growth factor for T cells, and its synthesis is increased after TCR activation. Inhibition of IL-2 synthesis is the target of the calcineurin inhibitors cyclosporin A (CsA) and FK506, a class of antirejection drugs in current use [91]. PD184352 inhibited IL-2 production induced by TCR activation by anti-CD3/anti-CD28 antibody coligation with an IC_{50} value of 47 nM [225]. Similar effects have been shown for a variety of the small-molecule MEK1 inhibitors [225]. Additionally, PD184352 suppressed T cell proliferation in the human mixed lymphocyte reaction (MLR) model with an IC_{50} value of 186 nM [225]. However, despite this encouraging *in vitro* data, *in vivo* data has thus far been disappointing. In the ear-neonatal heart transplant model, in which neonatal heart tissue from one mouse strain is transplanted into the pinna of another strain, treatment with PD198306 (2-(2-methyl-4-iodophenylamino)-N-cyclopropylmethoxy-3,4,5-trifluorobenzamide) shortened graft survival [225].

3.3.3 Treatment of septic shock with mitogen-induced extracellular kinase 1 inhibitors

Warner-Lambert (Pfizer) has also patented the use of MEK1 inhibitors in the treatment of septic shock [226]. *In vitro* data suggest that the Erk pathway may be important in some of the events likely to be involved in the pathogenesis of this disorder. LPS is a major factor in the progression of septic shock. Binding of LPS to macrophages leads to massive production of TNFα, IL-1β, and IL-6. All of these cytokines induce Erk activation [31,85-87], which underlies the rationale for targeting MEK1 in the treatment of septic shock as proposed in the patent [226]. However, no *in vivo* data exists as yet to support or refute the efficacy of blocking MEK1 signalling in septic shock.

3.3.4 Treatment of asthma with mitogen-induced extracellular kinase 1 inhibitors

The use of MEK1 inhibitors in the treatment of asthma was patented in 2000 [227]. Asthma is a chronic condition, in which increased airway response to various stimuli is associated with inflammation and airflow limitation. Pulmonary eosinophils, whose survival, differentiation and migration require IL-5, are believed to play a large role in the underlying pathology [92]. Splenocytes isolated from ovalbumin (OVA)-primed mice produce IL-5 *in vitro* when restimulated with OVA, and this was blocked in the presence of certain MEK inhibitors, including PD184386 (2-(2-chloro-4-iodophenylamino)-N-hydroxy-3,4-difluoro-5-bromobenzamide), PD171984 (2-(2-methyl-4-iodophenylamino)-N-hydroxy-3,4-difluoro-5-bromobenzamide) and PD184352 [227]. When transferred into naive recipient mice, the restimulated splenocytes induced eosinophilic lung inflammation. Again, this was almost completely inhibited if restimulation occurred in the presence of PD183486, PD171984 or PD184352 [227]. However, in a mouse model of antigen-induced eosinophilic lung inflammation induced by OVA-sensitisation followed by OVA aerosol challenge, only PD171984 was able to inhibit eosinophilic lung inflammation with a maximum effect of 55% when given 1 day prior to challenge. This effect was seen despite the lower potency of PD171984 as compared with PD184386 in blocking IL-5 production *in vitro* [227]. This raises the possibility that the effects of PD171984 in this model may be due to mechanisms other than MEK1 inhibition. No significant toxic effects of therapy were observed, but drug administration was short-term.

3.3.5 Mitogen-induced extracellular kinase 1 inhibitors as antiviral agents

An approach to antiviral treatment using MEK1 inhibitors has also been patented [228]. Since viral replication depends on the health of the host, particularly in the early stages of infection, two assays were used to select antiviral MEK1 inhibitors. The first assesses the inhibition of viral production (IC_{50}) and the second assesses host toxicity (TC_{50}) [228]. The ideal inhibitor will thus have a low IC_{50} with a high TC_{50}. It was found that the IC_{50}/TC_{50} values of PD120611 and PD177168 for human cytomegalovirus (HCMV) were 2.2μM/30μM and 0.8μM/9μM, respectively and for herpes simplex virus type 1 (HSV-1) were 6.9μM/13μM and 3.0μM/11μM, respectively [228], indicating that both of these compounds are better inhibitors of HCMV than of HSV-1. For HIV, four MEK1 inhibitors (PD185848, PD185625, PD198306, and PD203311) were found to have TC_{50} values that were 20 – 30 fold higher than the IC_{50} value (determined by thymidine incorporation) in assays carried out on macrophages [228]. When assayed on CD4 T cells, the difference between the IC_{50} and TC_{50} for these compounds was only small. All of the IC_{50} values reported for MEK1 inhibitors for any virus disclosed were in the micromolar range, which is 100 – 1000-fold higher than the IC_{50} value of these compounds for inhibiting MEK1 activation [206]. This suggests that the low efficacy of these compounds as antiviral agents is not directly related to their ability to inhibit MEK1.

3.4 Use of mitogen-induced extracellular kinase 1 inhibitors to treat chronic pain

Warner-Lambert (Pfizer) has claimed the use of specific classes of MEK1 inhibitors in the treatment of neuropathic and chronic inflammatory pain in several patents [229-232]. At least three signalling pathways have been implicated in afferent nociceptor sensitisation by inflammatory mediators, including PKA, PKC-ε and Erk [93]. The importance of Erk signalling

was shown by the effects of local injection of a constitutively active MEK1, which produced hyperalgesia unresponsive to treatment with PKA or PKC inhibitors [93]. Persistent inflammation produced by injection of complete Freund's adjuvant (CFA) into a hindpaw also caused sustained Erk activation in afferent spinal cord neurons. Intrathecal injection of U0126 inhibited Erk phosphorylation and attenuated the establishment and maintenance of hyperalgesia [94]. Two animal models of neuropathic pain include diabetic neuropathy (DN) induced by streptozotocin administration, and chronic constriction injury (CCI) of the sciatic nerve. The oral administration of a GABA agonist S-(+)-3-isobutylgaba (pregabalin) has been shown to be effective in reducing both static allodynia, as measured by paw withdrawal threshold in grams, and dynamic allodynia, as measured by light stroking of a hind paw [95]. In comparison, the MEK1 inhibitors PD198306, PD184352, and PD224552 were only effective for up to 2 h following intrathecal administration. Overall, pregabalin had greater efficacy with longer-lasting benefit. Neither oral nor local routes of administration of PD198306 had any significant effect [229].

4. Expert opinion

The Erk signalling cascade has multiple functions in central cellular processes. Identification of its deregulation in many human diseases is thus not surprising, and provides a potential target for disease intervention. This underlies the recent rapid development of patents dealing with MEK1 inhibitors.

4.1 Small-molecule mitogen-induced extracellular kinase inhibitors

Several companies have developed MEK1/2 inhibitors, although the PD series of compounds was patented primarily by Warner-Lamber (Pfizer). Due to the high homology between MEK1 and MEK2, with 90% identity in amino acid residues, most small-molecule MEK inhibitors patented should inhibit both MEK1 and MEK2 activation. The exception is PD98059, which is a more potent inhibitor of MEK1 [39]. Although the potencies of some quinoline derivatives (compounds 6c and 6d) are hard to assess, most quinoline compounds have a relatively low IC_{50} value (in the micromolar range) for inhibiting MEK activation, as do the benzenesulfonamides, sulphohydroxamic acid derivatives, and diarylamine derivatives. Together with the antisense MEK1 inhibitors, these compounds are unlikely to be useful either as tools to dissect Erk signal transduction pathways or as therapeutic agents.

The most potent MEK inhibitors, with IC_{50} values in the nanomolar or sub-nanomolar range, belong to the resorcyclic acid lactones, benzoheterocycles and the benzoic acid/benzhydroxamic acid derivatives. The resorcyclic acid lactones compete with ATP and it should be cautioned that their IC_{50} values depend on assay ATP concentrations. Compounds from the latter groups are non-competitive with ATP and are thus highly specific for MEK1/2 protein kinases. They provide the most commonly used MEK1 inhibitors for academic and clinical trial purposes (PD98059, U0126, and PD184352). These three MEK1 inhibitors also display higher affinity for unphosphorylated MEK1 than for activated MEK1 and inhibition of Raf-mediated MEK1 activation rather than blockade of MEK1 activity *in vivo* [41]. However, although U0126 did not inhibit MKK3, MKK4, JNK [39], MKK6 or MKK7 [40,41], there is a general lack of data concerning the activity of MEK1/2 inhibitors on related kinases including MKK3, 4, 5, 6, and 7.

4.2 Therapeutic potential of mitogen-induced extracellular kinase 1 inhibitors

Consistent with the activation of Erk in numerous cellular processes, patents and publications exist for a wide variety of diseases, including cancer, immunological and inflammatory disorders (arthritis, organ transplant rejection, septic shock, asthma, viral infection), ischaemia and chronic pain. While Erk functions in many cellular processes, this protein kinase is unlikely to be the central player in all of these conditions. It would thus be expected that MEK1 inhibitors would be most useful in diseases where disruption of Erk regulation has been shown to be of importance. To date, cancer therapy fits this construct most closely.

4.2.1 Targeting the mitogen-induced extracellular kinase-extracellular signal-regulated kinase (MEK-Erk) pathway in cancer

Although cancer results from the accumulation of multiple mutations, an initiating event is required for tumour formation [96]. Targeting initiation events prevent tumourigenesis and leads to tumour regression. Amplification of cyclin D1 was found in ~ 40% of human breast cancers [97] and cyclin D1-deficient mice were protected completely from Neu- and Ras-induced breast cancer, but not cancers initiated by Wnt and Myc [98]. Expression of H-Ras^{V12G} and K-Ras^{G12D} in mice initiated melanoma and lung adenocarcinomas, respectively. Prevention of their expression subsequently led to regression of both tumours [99,100]. Sustained expression of Myc in haematopoietic cells and keratinocytes resulted in the formation of lymphomas and papillomas, whilst inactivation of Myc subsequently led to their regression [101,102].

The most promising application of MEK1/2 inhibitors would thus be in the treatment of tumours in which hyperactivity of the MEK-Erk pathway plays a pathogenetic role. Although MEK inhibitors were found to be relatively inefficient in the treatment of both neurofibromatosis type 1 (NF1) [233] and implanted P388 leukaemia [77], they prevented growth and development of some colon cancers and melanomas [77,206,219]. Both of these tumours contain high levels of Erk activation [206,219]. Although PD184352 was unable to induce regression of implanted HT-29 colon cancer [77], inhibition of Erk activation led to regression of melanoma implanted in mice

[219], indicating that MEK inhibitors hold great promise in targeting melanoma. This is concordant with recent data showing that mutations in the B isoform of Raf kinase (BRAF) gene occur in 66% of malignant melanomas and in 88% of these the single substitution V599E, leading to increased Erk activation, was present [23]. Furthermore, in a limited trial of 150 patients with melanoma refractory to other cancer therapy, it was found that inhibition of Erk activation resulted in reduction of tumour size by > 50% in 66% of patients [219]. MEK inhibitors may be useful in the treatment of some breast cancers with Neu or Ras oncogene amplification, which result in Erk activation. This promotes G1 cell cycle progression, through cyclin D1 upregulation, and cyclin D1 null mice have been shown to be completely refractory to Neu and Ras-induced cancer [98]. Currently, PD184352, also known as CI-1040, is in both phase I and II clinic trials for the treatment of various cancers [103]. The level of phosphorylated Erk was examined by Western blot in PMA-stimulated mononuclear cells obtained from patients treated with CI-1040 (800 mg). This revealed that 15 days of treatment significantly lowered phosphorylated Erk [103].

With an IC_{50} value of 1 nM, PD184352 is one of the most potent MEK1 inhibitors. Several other compounds related to PD184352, as well as some of the resorcylic acid lactones and benzoheterocycles, have compatible IC_{50} values (Table 1). Their potential in cancer therapy has yet to be determined. PD184352 was suggested to inhibit Raf mediated MEK1 activation rather than to block MEK1 activity *in vivo* [41], consistent with the observation that PD184352 blocked colon cancer growth more effectively if applied at implantation rather than after established tumour growth [77]. Thus, reduction of basal level MEK activity by inhibitors of Ras/Raf or by resorcylic acid lactone MEK1/2 inhibitors before administration of PD185342, may improve cancer treatment efficacy. Since the resorcylic acid lactones inhibit MEK activity by competing with ATP [214], they should theoretically be able to reduce basal levels of activated MEK1/2. Cotargeting the MEK-Erk pathway in conjunction with compounds inhibiting survival pathways, or inducing DNA damage, has also been tested. The effectiveness of these combinations in cancer therapy is unknown. One should be very cautious in combining DNA-damaging agents with MEK inhibitors in cancer treatment, since Erk activity has been reported to play a role in DNA-damage induced cell cycle arrest and apoptosis [8].

Table 1. mitogen-induced extracellular kinase 1 inhibitors with potency compatible to that of PD184352

Compounds	IC_{50}	Patent Ref.
7-fluoro-6-(4-iodo-2-methyl-phenylamino-)-1H-benzoimidazole-5-carboxylic acid (PD205293)	14 nM	[215]
7-fluoro-6-(4-iodo-2-methyl-phenylamino-)-1H-benzoimidazole-5-carboxylic acid cyclopropylmethoxy-amide (PD254552)	< 10 nM	[215]
[5Z]-3,4,9,10,11,12-hexahydro-8,9,16-trihydroxy-14-3-methyl-1H-2-benzoxacyclotetradecin-1,7(8H)-dione (L-783277)	4 nM	[214]
3,4,-Difluoro-N-hydroxy-2-(4-iodo-2-methyl-phenylamino)-benzamide	1 nM	[206]
2-(2-Chloro-4-iodo-phenylamino)-4-fluoro-N-hydroxy-benzamide (HCL salt)	< 1 nM	[206]
5-Bromo-N-cyclopentylmethoxy-3,4,-difluoro-2-(4-iodo-2-methyl-phenylamino)-benzamide	8 nM	[206]
5-Bromo-N-cyclobutylmethoxy-3,4,-difluoro-2-(4-iodo-2-methyl-phenylamino)-benzamide	4 nM	[206]
2,3,5,-Trifluoro-6-(4-iodo-2-methyl-phenylamino)-4-(4-methyl-piperazin-1-yl)-benzoic acid methyleaster dihydrofluoride salt	2 nM	[204]
5-bromo-3,4-difluoro-2-(4-iodo-2-methyl-phenylamino)-benzoic acid	5 nM	[204]

4.2.2 *Use of mitogen-induced extracellular kinase 1 inhibitors in the treatment of arthritis and chronic pain*

MEK1/2 inhibitors show promise in the treatment of arthritis and chronic pain. RA is a chronic inflammatory disease in which TNFα is believed to play a major role. However, TNFα blockade is an ineffective therapy in approximately one-third of patients in clinical trials [104]. While TNFα-null mice could still be induced to develop arthritis similar to human RA, BALB/cA mice deficient in the IL-1 receptor antagonist (IL-1ra) spontaneously developed chronic inflammatory polyarthropathy, a disease resembling human RA [105,106]. This suggests a major role for IL-1 in human RA, and recombinant IL-1ra is currently in clinical trials. Several MEK inhibitors were found to potently block IL-1-induced MMP3 production with an IC_{50} value in the nanomolar range, as well as to inhibit IL-1-induced cartilage degradation by 75%. MEK inhibitors are thus promising new therapeutic agents for this disorder.

Recent developments reveal that Erk activity plays a major role in neuronal response to Ca^{2+} and cAMP signals [6]. This may support a role for Erk activation in delivering chronic pain signals to the CNS. The MEK inhibitor PD184352, delivered through intrathecal injection, was found to temporarily block pain as efficiently as pregabalin, although other modes of administration were largely ineffective [229].

4.2.3 *Use of mitogen-induced extracellular kinase 1 inhibitors in the treatment of other diseases*

MEK inhibitors have been patented to treat several other disease including ischaemia, asthma, septic shock, transplant rejection, and viral infection (e.g., HIV, human cytomegalovirus (HCMV), HSV). Since a virus utilises host cells for its replication, the health of the host is vital, especially in the early stages of infection. It is thus hard to envisage how MEK inhibitors that target host cells could selectively inhibit viral replication. The most commonly used immunosuppressants in organ transplantation are CsA, FK506, and rapamycin. While CsA and FK506 block TCR ligation-induced IL-2 production by

inhibiting calcineurin, rapamycin blocks IL-2-induced T-cell proliferation by inhibiting the TOR protein kinase [107], attesting to the importance of IL-2 blockade in this clinical setting. Although MEK inhibitors were found to potently inhibit TCR ligation-induced IL-2 production, they were unable to delay transplant rejection [225].

Although *in vivo* data for MEK1 inhibition in cerebral ischaemia models appears promising, with reduction of infarct volume by 43% and improvement in neurological function [223], it remains to be seen whether more delayed administration of an MEK1 inhibitor after the onset of ischaemia will be beneficial, thus making this more relevant to the treatment of stroke patients. In situations of cardiac arrest, early administration of an MEK1 inhibitor after ischaemic insult may be more clinically feasible. Caution must prevail, however, given the potential beneficial effect of Erk activation on cell responses in I/R. For example, during reoxygenation of endothelial cells (EC) after 5 h of ischaemia, MEK1 inhibition prevented EC barrier restoration [108]. Clinically, this may lead to worsening of cerebral oedema and neurological function after I/R. Similarly, in cardiomyocytes, MEK1 inhibitors prevented the protective effect of Erk activation against a second more potent ischaemic event [109].

Although MEK1/2 inhibitors were patented to have potential in treating asthma and septic shock, their efficacy appears to be relatively low [223,226,227]. No data were presented to support a role for MEK inhibitors in treating septic shock [226]. Although the MEK inhibitor PD184386 could block OVA-induced eosinophilic lung inflammation by 55% [227], long-term administration of MEK inhibitors should be approached with caution, particularly in young patients with asthma.

4.3 Cautions

Although an animal study and a Phase I clinical trial have revealed that PD184352 (CI-1040) is well tolerated [77,103], the side effects of long-term treatment with MEK inhibitors are not clear. In view of the multiple functions of the Erk pathway, side effects associated with long-term exposure to MEK inhibitors are likely. This will be a critical concern in using MEK inhibitors to target chronic disases.

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